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Effects of Dietary Aloe Vera on Growth Performance, Skin and Gastrointestine Morphology in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

This study investigated the effects of dietary Aloe vera on growth performance, some histological alterations in rainbow trout skin and gastrointestinal tract and disease resistance against *Streptococcus agalactiae* (*S. agalactiae*). Four treatments were designed including a control and Aloe vera incorporated in the fish feed at 0.01%, 0.1% and 1%, which were administrated for a period of six weeks. Results showed that Aloe vera at 0.1% and 1% administration resulted in improved specific growth rate (SGR) and feed conversion ratio (FCR). The Aloe vera-treated groups, improvement in proximal intestine, pyloric caeca and skin epidermis histology were also noted. The mortality rate after challenge with *S. agalactiae* was significantly low in fish fed 0.1% and 1% Aloe vera. The present study suggests that Aloe vera especially at 0.1% and 1% feed administration may enhance effectively the growth performance, gastrointestinal and skin morphology and resistance against *S. agalactiae* in rainbow trout

Keywords: Aloe vera, Rainbow trout, gastrointestine, *Streptococcus agalactiae,* histology.

Introduction

Stressful rearing conditions render the cultured fish highly sensitive to different diseases. Many studies have looked into the modulation of the immune system in fish to prevent disease outbreaks and enhance fish health and growth (Magnadóttir, 2006; Bowden, 2008). The optimal dose and duration of immunostimulants have promising roles in aquaculture by enhancing the resistance of cultured fish against diseases (Bricknell and Dalmo, 2005; Magnadóttir *et al.,* 2006). Some information suggests the effects of some herbs as natural immunostimulants with direct or indirect effects on fish skin and intestinal microflora and histology (Galina *et al.,* 2009; Merrifield *et al.,* 2011; Heidarieh *et al.,* 2012; Xueqin *et al.*, 2012).

Aloe barbadensis, Aloe vera, plant of the Lily family in warm and frost-free climates, has been known for centuries as a potent medicinal plant according the "folk medicines" of cultures around the world. Any Aloe vera liquid product, whether called gel, juice or whole leaf extract, comprises the fluid obtained by breaking up the structure of the Aloe leaf and separating off the solid residues to leave a more or less clear solution (Plaskett, 1998). Beneficial effects of Aloe vera in human and laboratory animals are contributed to the promotion of immune system, anti-inflammatory, pro-healing, gastrointestinal, antidiabetic and anti-arthritic effects (Plaskett, 1998). There is limited information available on the immunostimulatory, anti-toxicity and growth effects of Aloe in some fish species (Kim *et al.,* 1999; Alishahi *et al.,* 2010; Zodape, 2010; Wang *et al.,* 2011). To the best of our knowledge, there is no information in the literature on the role of dietary Aloe vera, in the prevention of infectious diseases and histological alterations to skin and gastrointestinal tract in rainbow trout. The aim of the present study was to investigate the effect of the Aloe vera on the growth, skin and intestinal histology and protection against pathogen *Streptococcus agalactiae* (*S. agalactiae*) in rainbow trout and to examine the optimal dose of this herb and duration of feeding.

Materials and Methods

Diets

Four diets based on a formulation of 38% protein, 18% lipid, 3% fiber, 0.7% phosphorous was prepared to contain different levels of Aloe vera gel

provided by Barij essential oil Co., Kashan, Iran. Aloe vera was incorporated into the diets as follows: no Aloe vera (control), 0.01% Aloe vera (group 1), 0.1% Aloe vera (group 2) and 1% Aloe vera (group 3). Aloe vera at different incorporation was added to palletized feed and then feed were separately dried in the oven at 30°C. Palletized feed were sent to oiling process in order to be covered with fish oil. Pellets were packed and stored in tightly sealed plastic bags at 8-10°C until they were used in the feeding experiments.

Fish and Experimental Conditions

360 rainbow trout (mean body weight: 50.3 ± 5.4 g) were obtained from a commercial fish farm in Karaj, Iran and transferred to fish disease laboratory at University of Tehran. Fish were randomly allocated between 12 tanks in triplicate at a density of 30 fish per 300 L aquarium and maintained in continuously aerated free-flowing dechlorinated fresh water. During the experiment, the following conditions were maintained: water temperature 15.5 ± 1.1˚C, dissolved oxygen concentration 7.3 ± 0.5 mg/l, pH 6.9 ± 0.4 and total hardness 164 ± 10 mg/l as CaCO3. After 14 days adaptation, fish in each group were fed one of the four different diets at a total daily rate of 2% body weight for six weeks.

Growth Performance

At the termination of the feeding trial, 5 fish in each tank were individually weighed 24 h after the last feeding. Specific growth rate (SGR) and feed conversion ratio (FCR) were calculated as indicators for growth performance (Heidarieh *et al*., 2012).

FCR = total feed given/total weight gain

SGR = $100 \times [(\ln W_f - \ln W_i)/\text{days}]$,

Where W_f is mean final weight and W_i is mean initial weight

Skin and Gastrointestinal Histological Parameters

After one, four and six weeks, 4 fish samples were randomly selected from each tank and anaesthetized with clove powder (200 mg/l). Skin samples were removed from the mid-body on the left side (the same place) from all the fish. In order to examine proximal intestine and pyloric caeca morphology, after dissection of midline in ventral surface, gut proximal section and pyloric caeca were gently removed. Samples were fixed in 10% buffered formalin for 48 h, dehydrated in alcohols and xylene and then embedded in paraffin. A 5 micron subsamples was then rehydrated in alcohols and stained with haematoxylin-eosin. Length and thickness of proximal intestinal villi, pyloric caeca folds and epidermal thickness were measured using a graded ocular lens. The percentage of goblet cells in fish proximal intestine, pyloric caeca and total goblet density per centimeter skin epidermis were also defined.

In Situ *S. agalactiae* Challenge

At the end of sixth week, the number of rainbow trout in all treatment groups was adjusted to 5 fish per each tank. Fish from control group were randomly assigned to two subgroups of 5 fish each, and distributed in 6 tanks. Fish were challenged by I.P. injection with 0.1 ml of a suspension of *S. agalactiae* (RTCC 2051) (1.71×10^7 cells/ml). In positive control subgroup, fish were I.P. injected with the same concentration of *S. agalactiae*. Fish in negative control subgroup were also challenged by I.P. injection with 0.1 ml of phosphate buffer saline (PBS). Dead and moribund fish were removed and examined microbiologically for up to 14 days (Sepahi *et al.,* 2012).

Statistical Analysis

All the measurements were made in triplicate. The results were subjected to analysis of variance (ANOVA) followed by least significant differences (Tukey) test. Correlation coefficients were significant with $P< 0.05$.

Result

After the 6 week feeding period, group 2 and 3 exhibited significantly lower FCR than those fed the control diet (Table 1). Moreover, SGR significantly enhanced when Aloe vera at 1% (group 3) was included in fish diet (Table 2).

Different histologicalal parameters in fish intestine after one, four and six weeks feeding of Aloe vera are shown in Table 1. Higher intestine villus was noted in group 2 and 3 at first week. However, group 1 and 2 showed higher villus length at fourth week. In group 2 and 3, narrower intestine villus was clear during first week. The higher number of mucous cells per cm was noted in groups 1 and 2 during the first week. On the other hand, during sixth week higher cell density were exhibited in group 2 and 3.

Mophological parameters in fish pyloric caeca after one, four and six weeks feeding of Aloe vera are presented in Table 3. The pyloric caeca showed that fold length was increased in all treatment groups at week first and fish in group 1 at week six during this study. Addition of Aloe vera during first week decreased the pyloric fold width in highest dose (group 3). Significant increase in mucous cell number for fish in group 2 and 3 were shown at fourth and sixth weeks.

As shown in Table 4, epidermal thickness for fish in group 2 and 3 were greater than control group during fourth and sixth weeks. Mucous cell number in epidermis was greater in rainbow trout administrated

Table 1. Growth performance in rainbow trout fed control diet (C), 0.1 g/kg Aloe vera (1), 1 g/kg Aloe vera (2) or 10 g/kg Aloe vera (3) for six weeks

Feed conversion ratio	Specific growth rate	Weight gain (g)	Groups
1.5 ± 0.25a	1.5 ± 0.17a	42.06 ± 1.03a	C
1.65 ± 0.29a	1.36 ± 0.18a	37.05 ± 0.57a	1
1.1 ± 0.07b	1.89 ± 0.08ab	57.27 ± 1.10ab	2
1 ± 0.09b	2.2 ± 0.16b	63.66 ± 2.30b	3

Data represents the average (n=12) ± SE. Different superscripts in each column are significantly different at $P < 0.05$.

Table 2. Intestine morphology in rainbow trout fed control diet (C), 0.1 g/kg Aloe vera (1), 1 g/kg Aloe vera (2) or 10 g/kg Aloe vera (3) after one, four and six weeks

Groups	Intestine villus length (µm)			Intestine villus width (µm)			Intestine villus goblet density (%)		
	Week one	Week four	Week six	Week one	Week four	Week six	Week one	Week four	Week six
C	383.3 ± 16.6^{a}	400.3 ± 28.8^{a}	433.3 ± 17.6^{a}	170.7 ± 15.2^{a}	130.2 ± 20.1^{a}	123.3 ± 14.5^{a}	24.1 ± 1.1^{a}	23.3 ± 0.8^{ab}	20.5 ± 1.1^{a}
1	483.3 ± 16.6^{ab}	516.6 ± 76.3^{b}	466.6 ± 72.6^{a}	146.6 ± 20.2^{ab}	140.6 ± 15.2^{a}	143.3 ± 8.8^{a}	20.4 ± 0.5^{b}	20.2 ± 0.5^{a}	23.6 ± 0.8^{a}
2	520.9 ± 62.4^{b}	590.8 ± 32.1^{b}	403.3 ± 37.5^{a}	116.6 ± 8.8^{b}	146.6 ± 12.1^{a}	150.2 ± 25.1^{a}	34.6 ± 0.8^{c}	35.2 ± 7.6^{b}	34.1 ± 0.5^{b}
3	556.6 ± 21.8^{b}	490.5 ± 20.8^{ab}	486.6 ± 18.5^{a}	113.3 ± 8.8^{b}	140.3 ± 17.3^{a}	143.3 ± 6.6^{a}	26.6 ± 0.5^{a}	29.3 ± 2.3^{ab}	40.6 ± 2.3^{c}

Data represents the average (n=12) ± SE. Different superscripts in each column are significantly different at $P < 0.05$.

Table 3. Pyloric fold morphology in rainbow trout fed control diet (C), 0.1 g/kg Aloe vera (1), 1 g/kg Aloe vera (2) or 10 g/kg Aloe vera (3) after one, four and six weeks

Groups	Pyloric fold length(µm)			Pyloric fold width (µm)			Pyloric fold goblet density (%)		
	Week one	Week four	Week six	Week one	Week four	Week six	Week one	Week four	Week six
C	493.3 ± 23.3^{a}	700.2 ± 57.7^{a}	600.6 ± 28.8^{a}	126.6 ± 8.8^{a}	110.2 ± 5.7^{a}	106.6 ± 14.5^{a}	25.2 ± 0.5^{a}	25.3 ± 0.6^{a}	21.3 ± 0.8^{a}
1	673.3 ± 14.5^{b}	770.4 ± 47.2^{a}	716.6 ± 60.1^{b}	110.1 ± 5.7^{ab}	110.9 ± 5.7^{a}	96.6 ± 12.0^{a}	27.1 ± 4.0^{a}	24.1 ± 0.5^{a}	21.6 ± 1.8^{a}
2	693.3 ± 34.8^{b}	660.1 ± 32.1^{a}	623.3 ± 20.8^{ab}	113.3 ± 8.8^{ab}	133.3 ± 8.8^{a}	123.3 ± 6.6^{a}	28.6 ± 0.6^{a}	32.6 ± 1.4^{b}	34.3 ± 0.3^{b}
3	700.1 ± 26.4^{b}	676.6 ± 14.5^{a}	646.6 ± 15.2^{ab}	96.6 ± 8.8^{b}	123.3 ± 8.8^{a}	116.6 ± 5.7^{a}	29.6 ± 0.3^{a}	39.2 ± 2.1^{c}	42.6 ± 1.4^{c}

Data represents the average (n=12) ± SE. Different superscripts in each column are significantly different at $P < 0.05$.

Table 4. Skin morphology in rainbow trout fed control diet (C), 0.1 g/kg Aloe vera (1), 1 g/kg Aloe vera (2) or 10 g/kg Aloe vera (3) after one, four and six weeks

Groups	Skin epidermis thickness (µm)			Skin epidermis goblet density (per cm)		
	Week one	Week four	Week six	Week one	Week four	Week six
C	226.6 ± 8.8^{a}	220.1 ± 15.2^{a}	236.6 ± 8.8^{a}	746.6 ± 8.8^{ab}	760.4 ± 5.7^{ab}	730.7 ± 5.7^{a}
1	223.3 ± 8.8^{a}	220.1 ± 5.7^{a}	220.1 ± 11.5^{a}	716.6 ± 12.0^{a}	716.6 ± 8.8^{a}	733.3 ± 8.8^{a}
2	230.1 ± 5.7^{a}	253.3 ± 3.3^{b}	290.4 ± 10.8^{b}	726.6 ± 14.5^{a}	846.6 ± 8.8^{b}	916.6 ± 44.1^{a}
3	220.0 ± 15.2^{a}	283.3 ± 8.8^{b}	336.6 ± 12.1^{c}	760.1 ± 5.7^{b}	1300.4 ± 57.7^{c}	2233.3 ± 88.2^{b}

Data represents the average (n=12) ± SE. Different superscripts in each column are significantly different at $P < 0.05$

with Aloe vera at 1% (group 4).

There was a main effect ($P < 0.05$) of week on the goblet cell density per villus in fish intestine received Aloe vera at 1% (group 3) (Figure 1). There was main effect of week on goblet cell density in pyloric fold and epidermis (Figure 2 and 3).

In fish administrated with 0.1% Aloe vera, the density of the goblet cells per villus was higher at sixth week compared with first week. Meanwhile, fish I group 3 showed higher goblet cell number at fourth and sixth weeks compared with first week. Skin thickness also showed a significant difference during

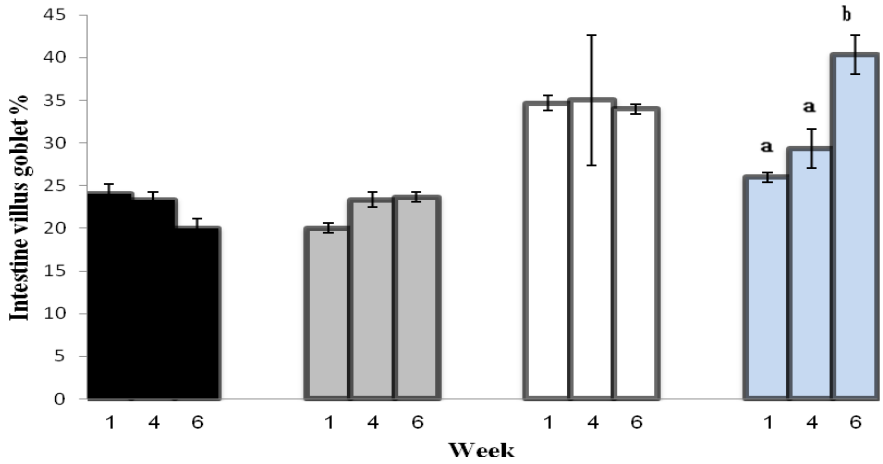

Figure 1. Intestine villus goblet cell density (%) in rainbow trout fed control diet ■ , 0.1 g/kg Aloe vera ▒ , 1 g/kg Aloe vera □ or 10 g/kg Aloe vera ▒ during first, fourth and sixth weeks. Data represents the average (n=12) ± SE. Different superscript alphabets in each treatment group are significantly different at $P < 0.05$

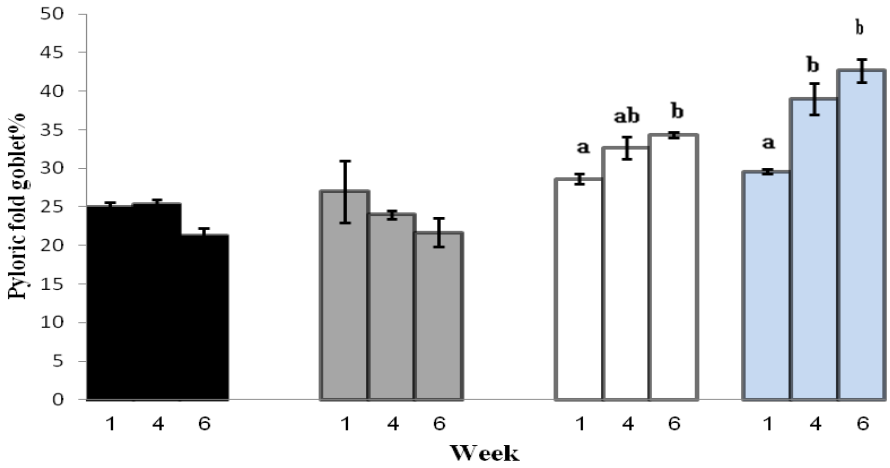

Figure 2. Pyloric fold goblet cell density (%) in rainbow trout fed control diet ■, 0.1 g/kg Aloe vera ▒ , 1 g/kg Aloe vera □ or 10 g/kg Aloe vera ▒ during first, fourth and sixth weeks. Data represents the average (n=12) ± SE. Different superscript alphabets in each treatment group are significantly different at $P < 0.05$.

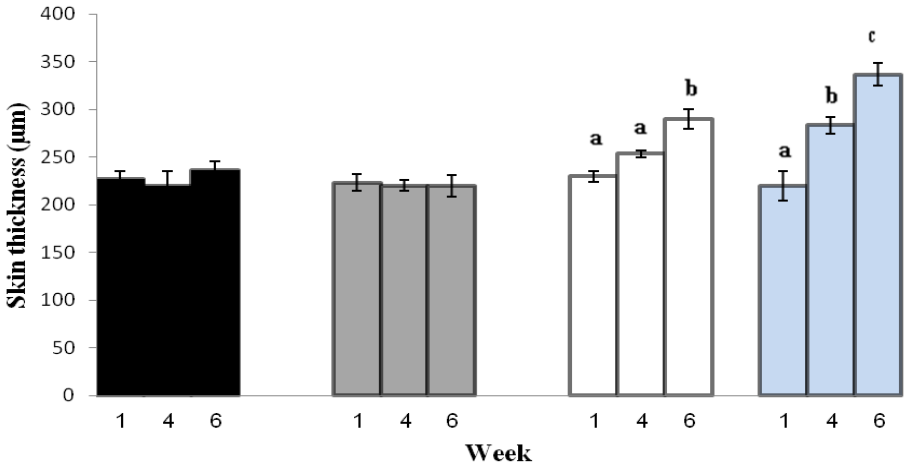

Figure 3. Skin thickness (µm) in rainbow trout fed control diet ■ , 0.1 g/kg Aloe vera ▒, 1 g/kg Aloe vera □ or 10 g/kg Aloe vera ▒ during first, fourth and sixth weeks. Data represents the average (n=12) ± SE. Different superscript alphabets in each treatment group are significantly different at $P < 0.05$.

different weeks especially in group 2 and 3 (Figure 4). Fish received 0.1% Aloe vera had higher skin thickness at sixth week compared with previous weeks while in fish fed 1% Aloe vera, at fourth and sixth weeks higher thickness were noted in comparison with first week.

The controlled exposure of rainbow trout to a virulent strain of *S. agalactiae* at the end of the feeding trial in group 2 and 3 resulted in limited mortality about 53% and 36% respectively. The highest percentage of mortality was observed in diet 1 (0.01% Aloe vera) with 83% of cumulative mortality. In control fish challenged with *S. agalactiae* and PBS, 70% and 0% cumulative mortality were observed (Figure 5).

Discussion

The present study demonstrated that high levels of Aloe vera (0.1% and 1%) had a positive effect on rainbow trout growth performance. Conversely, Aloe vera in different inclusion rates had no effect on *Acipenser baerii* (Wang *et al.*, 2011). Aloe vera is composed of 75 potentially active compounds: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids (Surjushe *et al.*, 2008). Improvement in growth performance by Aloe vera could be related to better nutrient digestibility and absorption, improved digestive enzymes and maintaining the function and structure of the small intestine, leading to an increased digestive capacity of

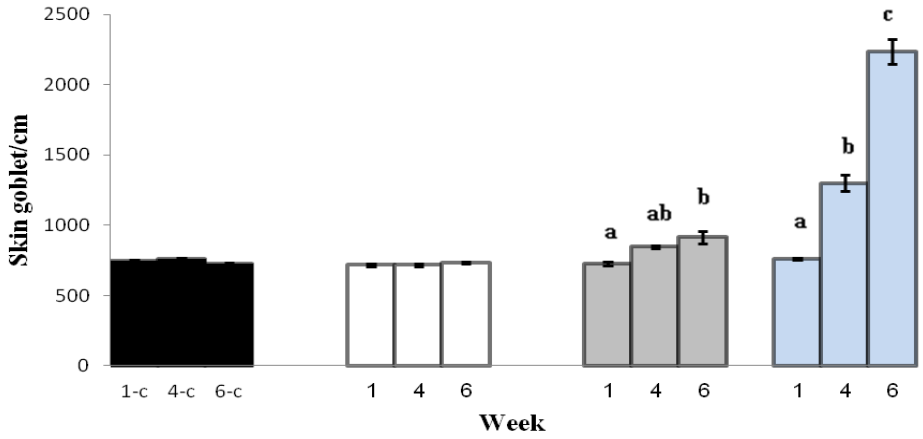

Figure 4. Skin goblet cell density (%) in rainbow trout fed control diet ■, 0.1 g/kg Aloe vera ■, 1 g/kg Aloe vera ■ or 10 g/kg Aloe vera ■ during first, fourth and sixth weeks. Data represents the average (n=12) ± SE. Different superscript alphabets in each treatment group are significantly different at $P < 0.05$.

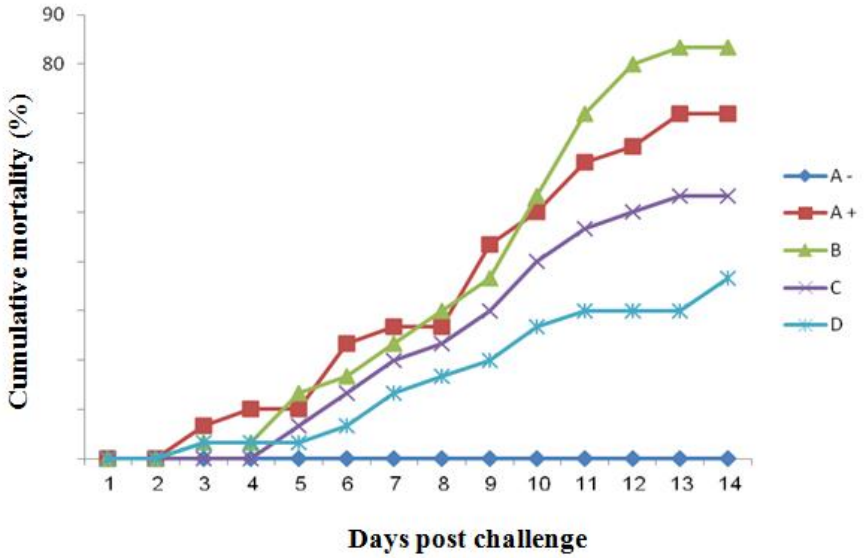

Figure 5. Cumulative mortality after *Streptococcus agalactiae* challenge in rainbow trout fed control diet, 0.1 g/kg Aloe vera, 1 g/kg *Aloe vera* or 10 g/kg Aloe vera: Group A- (0 g/kg Aloe vera, without bacterial challenge), group A+ (0 g/kg Aloe vera, bacterial challenge), group B (0.1 g/kg Aloe vera, bacterial challenge), group C (1 g/kg Aloe vera, bacterial challenge) and group D (10 g/kg Aloe vera, bacterial challenge).

the gut. In this study, improvement of gastrointestinal morphology was clearly observed.

The present study demonstrated that Aloe vera increased the villus length and decreased the villus width in fish intestine at different levels of administration. In pyloric caeca, which represents a way to increase intestinal surface area, higher and narrower folds were also shown. Similarly, following the feeding of different immunostimulants to fish species, increased villus height, fold height, enterocyte height, as well as reportedly augmenting surface area of the gut mucosa were observed (Yilmaz *et al.*, 2007; Ngamkala *et al.*, 2010; Heidarieh *et al.*, 2012). Numerous anatomical features determine the total absorptive surface area of the gastrointestinal tract. For example, taller, narrower, and regularly shaped villi and higher number of villi per unit area are indicators that the function of the intestinal villi is activated (Samanya and Yamauchi, 2002; Miles *et al.*, 2006). In general, these villi provided greater surface area for absorption of available nutrients. These facts suggest that the villus function might be activated after feeding dietary Aloe vera in rainbow trout.

In this study, large amount of goblet cells in fish epithelium after feeding Aloe vera were noted. Even though higher goblet cell density in fish skin and intestinal tract after challenge with infectious diseases have been widely studied (Buchmann and Bresciani, 1998; Ringø *et al.*, 2003) few studies showed the higher mucous cell density in fish received different immunostimulants (Ottesen *et al.*, 2000; Heidarieh *et al.*, 2012; Xueqin *et al.*, 2012). Mucous cell densities in skin epidermis and gastrointestinal epithelium form a viscous, hydrated blanket on the surface of the mucosa that act as a sensitive first line of immune defense parameter in fish (Ringø *et al.*, 2003; Xueqin *et al.*, 2012). Therefore, it can be assumed that enhancement of goblet cell density especially in higher doses of Aloe vera administration could result in higher defense mechanism against pathogens in rainbow trout.

Increased epidermal thickness and mucous cell number in higher doses of Aloe vera were shown. In previous study, following *Gyrodactylus* infection a rapid increased in epidermal thickness and a modest increased in number, but not size or composition, of mucous cells were shown in guppy, *Poecilia reticulate* (Gheorghiu *et al.*, 2012). Fast *et al*. (2002) also showed that rainbow trout had higher protease and lysozyme activities as well as thicker epidermis and more abundant mucous cells (mid body) than Coho salmon and Atlantic salmon. Therefore, thickness of the epidermis and the number of mucous cells could have direct effects on skin enzyme variation which results in higher disease resistance and protection against pathogens (Sheikhzadeh *et al.*, 2012).

Survival of rainbow trout to *S. agalactiae* challenge was significantly higher in the groups fed the Aloe vera at 0.1% and 1% compared to the control group. Surprisingly, fish given diet supplemented with 0.01% Aloe vera had significantly lower survival rate than fish given control diet. Other studies have also observed increased resistance against *Vibrio alginolyticus* and *Aeromonas hydrophila* in rockfish (*Sebastes schlegeli*) and common carp (*Cyprinus carpio*) fed diets supplemented with Aloe vera (Kim *et al.*, 1999; Alishahi *et al.*, 2010). Even though the increased resistance following the administration of Aloe vera might be due to the enhanced function of some non-specific immune parameters, different antiseptic agents namely lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur have also been shown in Aloe vera (Surjushe *et al.*, 2008). These components have inhibitory action on fungi, bacteria and viruses (Surjushe *et al.*, 2008).

Feeding strategies should be developed for each fish species with respect to immunostimulant dose and duration in order to acquire enhanced immune system (Dalmo and Bøgwald, 2008). Therefore, duration of Aloe vera feeding was analyzed to determine the histological changes after one, four and six weeks. Results showed that higher doses of Aloe vera (0.1% and 1%) significantly enhanced different histological parameters namely intestine and pyloric fold goblet cell density, epidermis goblet cell percent and skin thickness especially during the week four and six of administration in comparison with one week feeding. Therefore, it is apparent that feeding with the Aloe vera for four or more weeks is effective in better performance. In conclusion, rainbow trout fed Aloe vera at 0.1% and 1% for more than four weeks demonstrated greater effectiveness for growth performance, disease resistance and gastrointestinal morphology than lower dose.

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References

Alishahi, M., Ranjbar, M.M., Ghorbanpour, M., Peyghan, R., Mesbah, M. and Razi jalali, M. 2010. Effects of dietary Aloe vera on some specific and nonspecific immunity in the common carp (Cyprinus carpio). Int. J. Vet. Res., 4: 189-195.

Bowden, T.J. 2008. Modulation of the immune system of fish by their environment. Fish Shellfish Immun., 25: 373-383. doi: 10.1016/j.fsi.2008.03.017

Bricknell, I. and Dalmo, R.A. 2005. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immun., 19: 457-472. doi: 10.1016/j.fsi.2005.03.008

Buchmann, K. and Bresciani, J. 1998. Microenvironment of Gyrodactylus derjavini on rainbow trout Oncorhynchus mykiss: association between mucous

cell density in skin and site selection. Parasitol. Res., 84: 17-24. doi: 10.1007/s004360050350

Dalmo, R.A. and Bøgwald, J. 2008. ß-glucans as conductors of immune symphonies. Fish Shellfish Immun., 25: 384-396. doi: 10.1016/j.fsi.2008.04.008

Fast, M.D., Sims, D.E., Durka, J.F., Mustafa, A. and Ross, N.W. 2002. Skin morphology and humoral non-specific defence parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. Comp. Biochem. Physiol. A Comp. Physiol., 132: 645-657.

Galina, J., Yin, G., Ardo, L. and Jeney, Z. 2009. The use of immunostimulating herbs in fish an overview of research. Fish Physiol. Biochem., 35: 669–676. doi: 10.1007/s10695-009-9304-z

Gheorghiu, C., Marcogliese, D.J. and Scott, M.E. 2012. Waterborne zinc alters temporal dynamics of guppy Poecilia reticulata epidermal response to Gyrodactylus turnbulli (Monogenea). Dis. Aquat. Org., 98: 143–153. doi: 10.3354/dao02434

Heidarieh, M., Mirvaghefi, A.R., Akbari, M., Farahmand, H., Sheikhzadeh, N., Shahbazfar, A.A. and Behgar, M. 2012. Effect of dietary Ergosan on growth performance, digestive enzymes, intestinal histology, hematological parameters and body composition of rainbow trout (Oncorhynchus mykiss). Fish Physiol. Biochem., doi 10.1007/s10695-012-9602-8

Kim, K.H., Hwang, Y.J. and Bai, S.C. 1999. Resistance to Vibrio alginolyticus in juvenile rockfish (Sebastes schlegeli) fed diets containing different doses of aloe. Aquaculture, 180: 13–21. doi: 10.1016/S0044-8486(99)00143-X

Magnadóttir, B. 2006. Innate immunity of fish (overview). Fish Shellfish Immun., 20: 137-151. doi: 10.1016/j.fsi.2004.09.006

Magnadóttir, B., Gudmundsdottir, B.K., Lange, S., Steinarsson, A., Oddgeirsson, M., Bowden, T., Bricknell, I., Dalmo, R.A. and Gudmundsdottir, S. 2006. Immunostimulation of larvae and juveniles of cod, Gadus morhua L. J. Fish Dis., 29: 147–155. doi: 10.1111/j.1365-2761.2006.00701.x

Merrifield, D.L., Harper, G.M., Mustafa, S., Carnevali, O., Picchietti, S. and Davies, S.J. 2011. Effect of dietary alginic acid on juvenile tilapia (Oreochromis niloticus) intestinal microbial balance, intestinal histology and growth performance. Cell Tissue Res., 344: 135–146. doi: 10.1007/s00441-010-1125-y

Miles, R.D., Butcher, G.D., Henry, P.R. and Littell, R.C. 2006. Effect of Antibiotic Growth Promoters on Broiler Performance, Intestinal Growth Parameters, and Quantitative Morphology. Poult. Sci., 476-485.

Ngamkala, S., Futami, K., Endo, M., Maita, M. and Katagiri, T. 2010. Immunological effects of glucan and Lactobacillus rhamnosus GG, a probiotic bacterium, on Nile tilapia Oreochromis niloticus intestine with oral Aeromonas challenges. Fish Sci., 76: 833–840. doi: 10.1007/s12562-010-0280-0

Ottesen, O.H. and Olafsen, J.A. 2000. Effects on survival and mucous cell proliferation of Atlantic halibut, Hippoglossus hippoglossus L., larvae following microflora manipulation. Aquaculture, 187: 225-228. doi: 10.1016/S0044-8486(00)00314-8

Plaskett, L.G. 1998. The health and medical use of Aloe vera. Life Sciences Press. ISBN: 0-943685-21-4.

Ringø, E., Olsen, R.E., Mayhew, T.M. and Myklebust, R. 2003. Electron microscopy of the intestinal microflora of fish. Aquaculture, 227: 395–415. doi: 10.1016/j.aquaculture.2003.05.001

Samanya, M. and Yamauchi, K. 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comp. Biochem. Physiol. A. Comp. Physiol., 133: 95–104. doi: 10.1016/S1095-6433(02)00121-6

Sepahi, A., Heidarieh, M., Mirvaghefi, A.L., Rafiee, G.R., Farid, M. and Sheikhzadeh, N. 2012. Effects of Water Temperature on the Susceptibility of Rainbow Trout to Streptococcus agalactiae. Acta Acta Sci. Vet., 41: 1097.

Sheikhzadeh, N., Heidarieh, M., Karimi Pashaki, A., Nofouzi, K., Ahrab Farshbafi, M. and Akbari, M. 2012. Hilyses® fermented Saccharomyces cerevisiae, enhances the growth performance and skin non-specific immune parameters in rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immun., 32: 1083-1087. doi: 10.1016/j.fsi.2012.03.003

Surjushe, A., Vasani, R. and Saple, D.G. 2008. Aloe vera: A short review. Indian J. Dermatol., 53: 163–166. doi: 10.4103/0019-5154.44785

Wang, C., Xu, Q.Y., Xu, H., Zhu, Q., Zheng, Q.S. and Sun, D.J. 2011. Effects of aloe powder on the growth performance and plasma indices of sturgeon (Acipenser baeri Brandt). J. Shanghai Ocean. Univ. 04.

Xueqin, J., Kania, P.W. and Buchmann, K. 2012. Comparative effects of four feed types on white spot disease susceptibility and skin immune parameters in rainbow trout, Oncorhynchus mykiss (Walbaum). J. Fish Dis., 35: 127-135. doi: 10.1111/j.1365-2761.2011.01329.x

Yilmaz, E., Genc, M.A. and Genc, E. 2007. Effects of dietary mannan oligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, Oncorhynchus mykiss. Isr. J. Aquacult., 59: 182–188.

Zodape, G.V. 2010. Effect of Aloe vera Juice on Toxicity Induced by Metal (Chromium) in Labeo Rohita (Hamilton). J. Appl. Sci. Res., 6: 1788-1793.